Appendix A: Cross-Network PBMC Processing Worksheet v7.0

Note: The fields in this worksheet must be filled out by hand, using a pen.

Specimen Processing Laboratory: Protocol:

Participant ID (PTID/PID): Visit Number: Visit Type:

Collection Date: Collection Time:

Processing Start Date: Processing Start Time: Processed By (Initials):

Reagents	Manufacturer	Lot Number	Expiration Date
DMSO			
FBS			
WDR: HBSS or PBS (circle one)			
Cell Separation Tube (frit)			
Density Gradient Media			

	Volume in mL (record as X.Y)			Expiration Date
	CPS	DMSO	FBS	
CPS				1 working day (<18hrs)

Data to be Captured During Processing	Sample
Sample tube type (circle one or record "other" tube type)	ACD / HEP / EDT Other: _____
Blood condition (circle one or more; add comments on reverse as needed)	SAT/ HEM / CLT
Measured usable whole blood volume (to the nearest 0.1mL)	mL
Indicate processing method (circle one)	CSTFB / overlay / underlay
Counting Method: Name of specific instrument or manual count (record in field to right)	
Counting re-suspension volume of HBSS (or other WDR) **(V)** (record as X.Y)	mL
Cell count average concentration **(C)**	x 10^6 cells/mL
Total cell number **(T) = C x V**	x 10^6 cells
Calculate cell yield/mL of whole blood. (QC check)= (T/Usable Whole Blood Volume)	x 10^6 cells/mL
If ***T/A* ≥ N1***; CPS re-suspension vol ***(V1) = A*** If ***T/A < N1***; Calculate estimated CPS re-suspension vol. ***(V1)=(T/N1x10^6 cells/mL)(1mL)***	mL
Calculate final CPS re-suspension volume (***V_f***), (V1 rounded DOWN to nearest whole (X.0) mL)	mL
Calculate actual number of cells per vial. ***N2*** = ***(T/V_f)*** **x V2**; (V2=1 mL) ***Note:*** Do not store more than 50 million cells per vial	x 10^6 cells/vial
Print and QC LDMS Label content/barcodes (initials of person (s) performing QC)	
Frozen Date and Time (ddMMMyyyy /HH:MM) (Explain in comments section if not within **4 hours** of processing start time)	
Number of Cryovials actually frozen ***Note:*** Should be equal to final CPS re-suspension volume for 1mL aliquots (***V_f***) and **≤ (A)**	
Complete remaining LDMS entries including total cell count & frozen time (Initials)	

Note:* **A = The maximum number of aliquots required according to the protocol-specific Laboratory Processing Chart (LPC). Do not store more than this number of aliquots.

Appendix A: Cross-Network PBMC Processing Worksheet v7.0 (Page 2 of 2)

Note: The fields in this worksheet must be filled out by hand, using a pen.

Specimen Processing Laboratory:

PTID/PID:

Transfer of Cryovials to Freezer Storage Box	
Person who transferred cryovials to storage box locations assigned by LDMS	
Date (ddMMMyyyy)/time cryovials were transferred from controlled-rate freezing device to storage box. (Sample must be maintained at -80°C during transfer)	
Initial (Primary) Review (Initials/Date)	
Final (Secondary) Review (Initials/Date)	

Hemacytometer Counts	Total Count	Viable Cells	Non-Viable
Square #1 (cells/mm^2)			
Square #2 (cells/mm^2)			
Square #3 (cells/mm^2)			
Square #4 (cells/mm^2)			
Average Cell Count per Square (cells/mm^2)			
PBMC Dilution Factor (1:DF**)			
Hemacytometer Factor for cells/mL	10^4	10^4	10^4
Cell count concentration (C) = (Average Cells/mm^2)(DF)(10^4); convert to 10^6 cells/mL	Not applicable	x 10^6cells/mL	Not applicable
% viability = (Viable cells 4 squares/total cells 4 squares) (100)	Not applicable		Not applicable

***Note:* Dilution Factor (DF) = (parts cells + parts dilution fluid)/ parts cells

Automated Cell Counts (10^3/µl=10^6/mL)	Count #1
Cell Count (C) as cells x 10^6/mL	
PBMC Dilution Factor (1:DF***)	
Cell Concentration = (C)(DF)	x 10^6cells/mL
% viability (if applicable)	

****Note:* Dilutions for automated counters are extremely rare. If performing direct counts, enter a 1 in the DF box and complete the column.

Comments, protocol deviations, and additional information not captured elsewhere in this worksheet:

Note: **(A) =** The maximum number of aliquots required according to the protocol-specific Laboratory Processing Chart (LPC). Do not store more than this number of aliquots.

Cross-Network PBMC Processing Standard Operating Procedure

Appendix A: Cross-Network PBMC Processing Worksheet v7.0 ***Ex: N1 = 20 mil cells; A = 5 aliquots***

Note: The fields in this worksheet must be filled out by hand, using a pen.

Specimen Processing Laboratory: **Lab 398** Protocol: **313**

Participant ID (PTID/PID): **123-456789** Visit Number: **2.0** Visit Type: **vst**

Collection Date: **08AUG2024** Collection Time: **08:00**

Processing Start Date: **08AUG2024** Processing Start Time: **08:45** Processed By (Initials): **CN**

Reagents	Manufacturer	Lot Number	Expiration Date
DMSO	**Sigma**	**RNBM0548**	**18JAN2025**
FBS	**Peak**	**13G1212**	**18AUG2025**
WDR: HBSS or PBS (circle one)	**Gibco**	**2660057**	**30APR2026**
Cell Separation Tube (frit)	**Greiner**	**E220337Q**	**14MAR2027**
Density Gradient Media	**Cytiva**	**1Q345061**	**31AUG2026**

	Volume in mL (record as X.Y)			Expiration Date
	CPS	DMSO	FBS	
CPS **Prepared 19AUG2024 08:30 CN**	**9.0**	**0.9**	**8.1**	1 working day (<18hrs)

Data to be Captured During Processing	Sample
Sample tube type (circle one or record "other" tube type)	ACD / HEP / EDT Other: _____
Blood condition (circle one or more; add comments on reverse as needed)	SAT/ HEM / CLT
Measured usable whole blood volume (to the nearest 0.1mL)	**86.3** mL
Indicate processing method (circle one)	CSTFB / overlay / underlay
Counting Method: Name of specific instrument or manual count (record in field to right)	**Manual Count**
Counting re-suspension volume of HBSS (or other WDR) **(V)** (record as X.Y)	**17.0** mL
Cell count average concentration **(C)**	**7.2** x 10^6 cells/mL
Total cell number **(T) = C x V**	**122.4** x 10^6 cells
Calculate cell yield/mL of whole blood. (QC check)= (T/Usable Whole Blood Volume)	**1.4** x 10^6 cells/mL
If ***T/A* ≥ N1***; CPS re-suspension vol ***(V1) = A*** If ***T/A < N1***; Calculate estimated CPS re-suspension vol. ***(V1)=(T/N1x10^6 cells/mL)(1mL)***	**5.0** mL
Calculate final CPS re-suspension volume (***V_f***), (V1 rounded DOWN to nearest whole (X.0) mL)	**5.0** mL
Calculate actual number of cells per vial. ***N2 = (T/V_f) x V2***; (V2=1 mL) ***Note:*** Do not store more than 50 million cells per vial	**24.4** x 10^6 cells/vial
Print and QC LDMS Label content/barcodes (initials of person (s) performing QC)	**CN**
Frozen Date and Time (ddMMMyyyy /HH:MM) (Explain in comments section if not within **4 hours** of processing start time)	**08AUG2024**
Number of Cryovials actually frozen ***Note:*** Should be equal to final CPS re-suspension volume for 1mL aliquots (***V_f***) and **≤ (A)**	**5**
Complete remaining LDMS entries including total cell count & frozen time (Initials)	**CN**

Note:* **A = The maximum number of aliquots required according to the protocol-specific Laboratory Processing Chart (LPC). Do not store more than this number of aliquots.

Example #1:
N1 = 20x10^6 cells/mL
A = 5 aliquots

Calculations:
CPS re-suspension volume (V1)

122.4/5 = 24.4>20

Thus, T/A ≥ N1

(V1) = A

Actual number of cells per vial (N2)

122.4/5 x 1 = 24.4x10^6 cells/vial

HIV/AIDS Network Coordination

Cross-Network PBMC Processing
Standard Operating Procedure

Appendix A: Cross-Network PBMC Processing Worksheet v7.0 ***Ex: N1 = 10 mil cells; A = 5 aliquots***

Note: The fields in this worksheet must be filled out by hand, using a pen.

Specimen Processing Laboratory: **Lab 398** Protocol: **313**

Participant ID (PTID/PID): **123-456789** Visit Number: **2.0** Visit Type: **vst**

Collection Date: **08AUG2024** Collection Time: **08:00**

Processing Start Date: **08AUG2024** Processing Start Time: **08:45** Processed By (Initials): **CN**

Reagents	Manufacturer	Lot Number	Expiration Date
DMSO	**Sigma**	**RNBM0548**	**18JAN2025**
FBS	**Peak**	**13G1212**	**18AUG2025**
WDR: HBSS or PBS (circle one)	**Gibco**	**2660057**	**30APR2026**
Cell Separation Tube (frit)	**Greiner**	**E220337Q**	**14MAR2027**
Density Gradient Media	**Cytiva**	**1Q345061**	**31AUG2026**

	Volume in mL (record as X.Y)			Expiration Date
	CPS	DMSO	FBS	
CPS **Prepared 19AUG2024 08:30 CN**	**9.0**	**0.9**	**8.1**	1 working day (<18hrs)

Data to be Captured During Processing	Sample
Sample tube type (circle one or record "other" tube type)	ACD / HEP / EDT Other: _____
Blood condition (circle one or more; add comments on reverse as needed)	SAT / HEM / CLT
Measured usable whole blood volume (to the nearest 0.1mL)	**46.3** mL
Indicate processing method (circle one)	CSTFB / overlay / underlay
Counting Method: Name of specific instrument or manual count (record in field to right)	**Manual Count**
Counting re-suspension volume of HBSS (or other WDR) **(V)** (record as X.Y)	**9.0** mL
Cell count average concentration **(C)**	**4.2** x 10^6 cells/mL
Total cell number **(T) = C x V**	**37.8** x 10^6 cells
Calculate cell yield/mL of whole blood. (QC check)= (T/Usable Whole Blood Volume)	**0.8** x 10^6 cells/mL
If ***T/A* ≥ N1***; CPS re-suspension vol ***(V1) = A*** If ***T/A < N1***; Calculate estimated CPS re-suspension vol. ***(V1)=(T/N1x10^6 cells/mL)(1mL)***	**3.7** mL
Calculate final CPS re-suspension volume (***V_f***), (V1 rounded DOWN to nearest whole (X.0) mL)	**3.0** mL
Calculate actual number of cells per vial. ***N2*** = ***(T/V_f)*** **x V2**; (V2=1 mL) ***Note:*** Do not store more than 50 million cells per vial	**12.6** x 10^6 cells/vial
Print and QC LDMS Label content/barcodes (initials of person (s) performing QC)	**CN**
Frozen Date and Time (ddMMMyyyy /HH:MM) (Explain in comments section if not within **4 hours** of processing start time)	**08AUG2024**
Number of Cryovials actually frozen ***Note:*** Should be equal to final CPS re-suspension volume for 1mL aliquots (***V_f***) and **≤ (A)**	**3**
Complete remaining LDMS entries including total cell count & frozen time (Initials)	**CN**

Note:* **A = The maximum number of aliquots required according to the protocol-specific Laboratory Processing Chart (LPC). Do not store more than this number of aliquots.

Example #2:

N1 = 10x10^6 cells/mL
A = 5 aliquots

Calculations:

CPS re-suspension volume (V1)

37.8/5 = 7.56<10

Thus, T/A < N1

(V1) = 37.8/10x10^6 cells/mL)(1mL)

Actual number of cells per vial (N2)

37.8/3 = 12.6x10^6 cells/vial